AN EXPERIMENTAL STUDY OF [illegible] IMMUNIZATION WITH LIVE VACCINE AGAINST Q FEVER

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AN EXPERIMENTAL STUDY OF ENTERAL IMMUNIZATION WITH LIVE VACCINE AGAINST Q-FEVER

[Following is the translation of an article by A. A. Vorobyev and V. N. Pautov, appearing in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 8, 1964, pages 41--45. It was submitted on 21 Dec 1963. Translation performed by Sp/6 Charles T. Ostertag Jr.]

Numerous epidemiological observations conclusively testify to the possibility of the infection of man and animals by the causative agent of Q-fever by the alimentary route. Under experimental conditions Zotov et al., Lashkevich, Babudieri and Moscovici, Combiescu et al., Fonseca et al., Gillespie and Baker, Philip, Ravaioli, Sobeslavsky and Syrucek, Winn et al., Winn and Elson, and other investigators demonstrated the possibility of infecting guinea pigs, monkeys, sheep, goats, calves, horses, cats, dogs, piglets, hens, parrots and pigeons by feeding them food containing virulent Rickettsia burneti.

Considering the susceptibility of man and various animals and birds to the alimentary introduction of virulent Rickettsia burneti, we undertook a study of the possibility of enteral immunization against Q-fever with a vaccine strain of Rickettsia burneti.

For the live vaccine against Q-fever we used strain M [Synonym for strain M-44] of Rickettsia burneti (Genig, 1960; Zdrodovskiy and Genig, 1962; Polozov and Pautov, 1961; Rybkina, 1960), obtained by Zdrodovskiy at the Department of typhus and other rickettsial diseases of the Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR, in the form of a 1% suspension, freeze dried on skimmed milk, from the infected vitelline membrane of chick embryos of the 67th passage. In the experiments we used an egg culture of the second subpassage, prepared in a solution of saccharose with 0.1% agar and 1.25% gelatin, which contained 10^7 ED50 of rickettsiae in 1 ml. [ED50 -- the median dose which induces the reaction in question.] For the ED50 we used a dose of rickettsiae which would ensure the formation of complement-fixing antibodies in a titer of no less than 1:10 in 50% of white rats. These rats weighed 25--30 g and had each received intraperitoneally 1 ml of successive 10-time dilutions of the culture. The calculation of the ED50 was done by Karber's method with Ashmarin's modification.

The live vaccine was diluted with skimmed milk and in doses of 1 ml was introduced through a special probe by small portions into the esophagus of guinea pigs which weighed around 300 g. The body temperature of the pigs was taken daily. After various periods of time the titer of the complement-fixing antibodies was determined in the blood serum of each animal. For setting up the reaction we used 4 units of antigen of the 2nd phase variant of Rickettsia burneti and an initial serum dilution of 1:10. Fixation of complement was carried out at 37° for an hour by the method described by Zdrodovskiy and Golinevich.

When testing susceptibility of immunized guinea pigs to the causative agent of Q-fever, 10^5 ED50 of virulent Rickettsia burneti was introduced into each of the animals intraperitoneally. In all the tests we used the same series of dry culture, which was a 10% suspension, vacuum dried from a frozen state, of yolk membranes of infected chick embryos in a 10% solution of saccharose with 0.1% agar and 1.25% gelatin (Pautov and Polozov, 1961). The same dose of Rickettsia burneti was administered to unvaccinated control animals. The temperature was measured in all the animals for a period of 15 days following infection. The absence of symptoms of the disease in the previously immunized animals along with the presence of a clear clinical picture of Q-fever in all the control pigs served as the criteria for the effectiveness of the vaccination.

In the first series of tests we investigated the susceptibility of guinea pigs to the enteral introduction of various quantities of strain M by using successive 10-time dilutions of the initial culture on milk. The results of the test, which are presented in table 1, testify to the high sensitivity of guinea pigs to the alimentary introduction of vaccine strain M of Rickettsia burneti. The dose of strain M which guaranteed the emergence of complement-fixing antibodies in 50% of the animals turned out to be equal to 60 ED50.

As is apparent from table 2, following the enteral introduction of 10^4 and 10^6 ED50 of strain M the titer of complement-fixing antibodies was relatively low. In the majority of animals on the 20--30th day it reached 1:20 -- 1:30, and only in individual pigs -- 1:160 -- 1:640. By the 90th day the titer of antibodies was significantly lowered, while in many of the pigs antibodies could not be detected. Thus, with the enteral introduction of strain M the same regularity was noted as with the subcutaneous vaccination.

During the investigation of the animals which were immunized by the enteral route no clinical symptoms of the disease were apparent, particularly the febrile reaction, even with the administration of 10^5 -- 10^6 ED50 of vaccine strain M.

After the enteral introduction of 10^6 ED50 of a virulent culture of Rickettsia burneti, an expressed form of Q-fever was noted in all the animals in 4--6 days. After 30 days, in the serum of all the pigs the antibody titer to the 2nd phase component of Rickettsia burneti reached 1:1280 -- 1:2560, after 60 days -- 1:320, and on the 90th day, 1:40 -- 1:160. After 90 days

the animals turned out to be non-susceptible to 10^5 ED50 of a virulent culture of rickettsiae.

The oral administration of 1 ml of a suspension of killed Rickettsia burneti (killed corpuscular vaccine), comprising 250 million microbial cells based on the optical standard, did not ensure the appearance of complement-fixing antibodies even in one of the pigs over a period of 60 days. After 60 days the animals turned out to be susceptible to a virulent culture of Rickettsia burneti.

It is shown in table 3 that with the subcutaneous and enteral administration of the same dose of strain M (10^6 ED50), in 20 and 30 days the content of complement-fixing antibodies was somewhat higher in the pigs vaccinated subcutaneously, though on the 30th day significant differenced in the average geometric titers of antibodies in animals from the stated groups were not noted (1:64 and 1:40 respectively). After 90 days all the animals, in whose serum antibodies were detected, regardless of titer proved to be resistant to 10^5 ED50 of virulent Rickettsia burneti.

In 3 tests on 83 pigs the susceptibility to a virulent culture of Rickettsia burneti in 60--90 days following the enteral immunization with live vaccine was tested. The results of the investigations are summed up in table 4. It turned out that all 65 pigs, in whose serum antibodies were found on the 20--30th day, regardless of the earlier introduced dose of vaccine strain M did not react to control infection with 10^5 ED50 of virulent Rickettsia burneti. On the contrary, in all 18 guinea pigs, in whose serum antibodies were not detected on the 20--60th day following immunization, the control infection caused the development of an expressed form of Q-fever, analogous with the disease in 16 non-immunized animals.

The results of the investigations testified to the effectiveness of enteral immunization of guinea pigs against Q-fever with live vaccine (strain M of Rickettsia burneti) and make it possible to propose the feasability of using this vaccine for the enteral immunization of humans.

Conclusions

1. The high susceptibility of guinea pigs to the enteral administration of vaccine strain M of Rickettsia burneti has been established.

2. The enteral administration of 60 ED50 (intraperitoneal) of vaccine strain M ensured the immunization of 50% of the guinea pigs.

3. In the serum of guinea pigs, by the 20--30th day following enteral immunization of 10^4 ED50 and by the 10--20th day when using 10^6 ED50 of strain M, complement-fixing antibodies appeared to the 2nd phase component of Rickettsia burneti.

4. Guinea pigs, in whose serum complement-fixing antibodies were contained on the 20--30th day following enteral immunization, were non-susceptible to 10^5 ED50 of a virulent culture of Rickettsia burneti after 60--90 days.

5. It is expedient to study the effectiveness of enteral immunization of persons against Q-fever with preparations of live vaccine of Rickettsia burneti (strain M).

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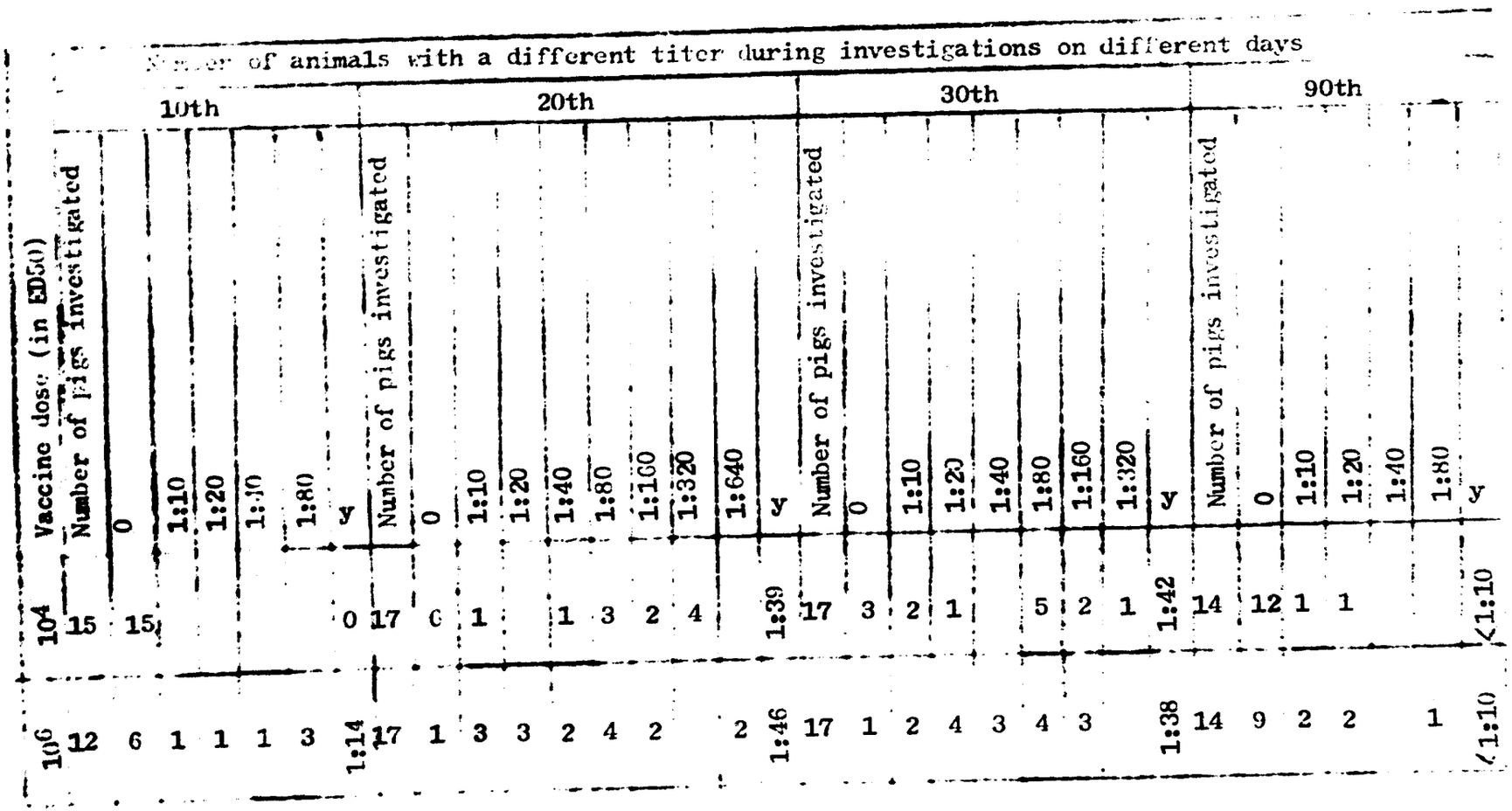
[Following is the English summary that appears with the Russian article.]

Guinea pigs are highly sensitive to enteral administration of the vaccinal M strain of Rickettsia burnetii. Enteral administration of 60 I U_{50} [Evidently should be ID] of M strain immunized 50 per cent of guinea pigs under the experiment. It is considered expedient to study the efficacy of enteral immunization of man against Q-fever with the live Rickettsia burnetii vaccine of M strain.

Table 2

Dynamics of the titer of complement-fixing antibodies to the antigen of the 2nd phase variant of Rickettsia burneti in the serum of guinea pigs, immunized by the enteral route with the live vaccine of Q-fever (summary of two tests).



Legend: y -- Average geometric titer of complement-fixing antibodies.

Table 4

Susceptibility of guinea pigs after enteral immunization with live vaccine (strain M) to a virulent culture of Rickettsia burneti (summary of 3 tests).

Dose of vaccine (in ED50), administered for immunization	Number of immunized pigs	Number of pigs	
		containing antibodies on the 20--30th day	not containing antibodies on the 20--30th day
1	5	0/1	4/4
10	4	0/2	2/2
10^2	10	0/6	4/4
10^3	3	0/2	1/1
10^4	45	0/38	7/7
10^5	3	0/3	-
10^6	13	0/13	-
All told . . .	83	0/65	18/18
Control -- guinea pigs not vaccinated earlier	-	-	16/16

Designations: Numerator -- number of pigs stricken with Q-fever after a control infection; Denominator -- number of pigs in group.

Table 1

Presence of complement-fixing antibodies in the serum of guinea pigs following the enteral introduction of live vaccine against Q-fever (strain M of Rickettsia burneti).

Dose of vaccine (in ED50)	Day serum investigated				Number of of pigs with antibodies from the total animals in group
	20th	30th	45th	60th	
1	0/6	1/6	0/6	0/6	1/6
10	1/5	2/5	2/5	0/5	2/5
10^2	4/5	4/5	4/5	1/5	4/5
10^3	4/6	4/6	3/6	1/6	4/6
10^4	3/4	3/4	3/4	0/4	3/4
10^5	5/6	6/6	3/3	1/3	6/6
10^6	5/5	5/5	4/4	1/4	5/5

Legend: Numerator -- number of pigs containing complement-fixing antibodies in a titer of no less than 1:10; Denominator -- number of pigs in group.

Table 3

Titers of complement-fixing antibodies in the serum of guinea pigs, immunized by the subcutaneous and enteral routes with live vaccine to Q-fever (strain M).

Method of immunization	Number of animals	Titer of complement-fixing antibodies on various days															
		20th								30th							
		0	1:10	1:20	1:40	1:80	1:160	1:320	1:640	y	0	1:10	1:20	1:40	1:80	1:160	1:320	y
Subcutaneous	12			1		7	4			1:90			1	3	7	1		1:64
Enteral	12	1	2	2	1	4			2	1:48	1	2	1	3	3	2		1:40

Each of the animals received 10^6 ED50 of live vaccine (strain M).